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# **Evaluation of the effects of different variables over the properties of plantain starch-based films and their potential application in packaging**

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Bogotá, Colombia

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# **Evaluation of the effects of different variables over the properties of plantain starch-based films and their potential application in packaging**

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Tesis de investigación presentada como requisito parcial para optar al título de:  
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*“Nothing in life is to be feared, it is only to be understood.  
Now is the time to understand more, so that we may fear less.”  
Marie Curie*

*To my parents, without them, nothing would be  
possible.*

*To my love, Manuel*



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## Resumen

La generación de residuos de la industria de plásticos de un solo uso ha motivado la búsqueda de materiales biodegradables que suplan la función de estos. Las películas derivadas de almidón pueden representar una solución a este problema. En este trabajo se realizó el aislamiento de almidón de plátano (*Musa paradisiaca*), de la variedad dominico hartón, a partir de residuos de segundas y terceras calidades de plátanos en estado verde en plantaciones de Casanare. Las películas se realizaron mediante la técnica *solvent casting*, usando agua, glicerina y ácido cítrico como plastificantes. Se caracterizaron las propiedades del almidón, de las soluciones gelatinizadas, y se evaluó el efecto de la interacción entre el almidón, la glicerina y el ácido cítrico sobre las propiedades mecánicas y de barrera de las películas de almidón. Los gránulos de almidón presentaron forma lenticular y temperatura de gelatinización de  $78.0 \pm 1.0$  °C. La adición de ácido cítrico disminuyó la viscosidad de las soluciones gelatinizadas mientras que la adición de glicerina no produjo cambios significativos en la misma. Incrementos en la concentración de ácido cítrico y glicerina disminuyeron el módulo elástico y la fuerza de tensión, mientras que aumentaron la permeabilidad al vapor de agua. Se evaluó la degradabilidad de las películas mediante termogravimetría, se encontraron porcentajes de degradabilidad desde 71% hasta 85%.

**Palabras clave:** almidón de plátano, plastificantes, películas de almidón

## Abstract

The generation of waste from the single-use plastics industry has motivated the search for biodegradable materials to replace the function of these. Starch-based films may represent a solution to this problem. In this work, we isolated plantain starch (*Musa paradisiaca*), of the variety Dominican hartón, from the residues of second and third qualities of plantains in a green state in plantations in Casanare. The films were made by the solvent casting technique, using water, glycerin, and citric acid as plasticizers. The properties of starch and gelatinized solutions were characterized, and the effect of the interaction between starch, glycerin, and citric acid on the mechanical and barrier properties of starch films was evaluated. Starch granules presented lenticular shape and gelatinization peak temperature of  $78.0 \pm 1.0$  °C. The addition of citric acid decreased the viscosity of the gelatinized solutions, while the addition of glycerin did not produce significant changes in viscosity. Increases in the concentration of citric acid and glycerin decreased the elastic modulus and tensile strength while increasing the Water Vapour Permeability (WVP). The degradability of the films was evaluated by thermogravimetry. Degradability percentages were found from 71% to 85%.

**Keywords:** plantain starch, plasticizers, starch-based films

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# Introduction

The starch is considered the most important carbohydrate in the human diet. It is produced by many plants as energy storage and can be found in its seeds, roots, tubers, fruits, stems, and leaves. It is a natural polymer composed of amylose (linear) and amylopectin (branched) chains, the latter gives it a semi-crystalline structure (Nakamura, 2015). These chains are densely packed in granules that are insoluble in water at room temperature. Depending on the botanical source, properties such as molecular structure, size, shape, and composition of the granules are different. This wide range of features allows different applications, for example, in the food industry and as substitute of fossil fuels in applications such as plastics, detergents, and adhesives. This fact is of utmost importance since it is a renewable and biodegradable raw material (Bemiller & Whistler, 2009).

The study of starch as a biofilm has acquired importance in recent years since the replacement of non-biodegradable materials has become more necessary every day (Oliveira et al., 2013). As a biodegradable material, starch is a very attractive, easily accessible, renewable, and cost-effective raw material for potential bio-based products. In its granular form, this polysaccharide can be isolated by sedimentation, centrifugation, and filtration, so its extraction can be carried out at a very low cost (Bemiller & Whistler, 2009). Additionally, some of its properties can be improved by physical, chemical, and enzymatic modifications of its structure which increases its potential for specific applications. However, starch-based biofilms also have some disadvantages compared with conventional plastics, such as high water-solubility, high water-absorption, rapid degradation, and poor mechanical properties. Those limitations have prevented these films of being exploited in the packaging industry (Valero-Valdivieso et al., 2013).

Despite of being a polymeric material, starch does not exhibit plastic properties, and therefore plasticizers and reinforcements must be used (Versino et al., 2016). Water is one of the most suitable and commonly employed plasticizers. Under the action of heat, it

induces a decrease in the viscosity of the solution by increasing the molecular mobility, through the reduction of the amount of hydrogen bonds between polymer chains, increasing the elasticity of the film (Cova et al., 2009). Also, the addition of water and heat modifies its crystalline structure resulting in a thermoplastic starch (TPS). There are other plasticizers that can also be used. For instance, glycerol has high penetration to the starch granules and improves the film flexibility. Citric acid is also an useful plasticizer, as it generates crosslinks in the polymer matrix, improving tensile strength, thermal stability, and additionally decreases the solubility of biofilms in water (Zhang et al., 2005)(Reddy & Yang, 2010).

The interest on developing TPS at industrial level began more than 30 years ago and there are now some companies in the world dedicated to it. One of the first to achieve this was Novamont, an Italian company founded in 1989 (Glenn et al., 2014). Novamont developed its product Maste-Bi, a corn-starch-based pellet with a wide range of applications, and has about 1000 product patents related to TPS products, among which, between 40% and 70% of starch is used (Novamont, 2020). Other companies have been developed, for example in Germany, Biotec, a company that uses up to 67% starch in its formulations and has about 250 TPS product patents (Biotec, 2020). In the United States, BASF, Cargill, KTM Industries, Cereplast and others have TPS products lines (Glenn et al., 2014).

In Colombia there is no consolidated market in TPS yet, but there have been significant advances in bioplastics mostly based on cassava starch (Navia et al, 2015). Colombia has a tremendous agricultural potential, and there are still many starches that can be studied for obtaining biofilms. Among them, the plantain, which is the fourth most important crop in the world and the second one in Colombia (4.4 Millions of tons cropped as of 2019), represent a good alternative as source of starch (Ministerio de Agricultura y Desarrollo rural, 2020).

Plantain starch is considered a low-cost raw material since it can be extracted from unripe plantains that cannot be sold due to size and quality classification and, are considered as waste by the farmers. Given its high potential, this starch presents great opportunities for future development, thus leaving the door open for the creation of innovative products (Molavi et al, 2015). Thereby, many efforts are made driving research towards scale-up of

process involving starch-based materials to a pilot plant, which can then be used on an industrial scale.

In this work, plantain (*Musa Paradisiaca*-Dominico Harton) was used as the botanical source of starch. This variety has been reported to have a starch content around 60% (Zhang et al., 2005). Plantain starch films were prepared by the solvent casting technique, which consists in dissolving the polymer in a solvent and spreading this mixture on a support, allowing the solvent to evaporate and obtaining the polymer film. The films were made using water, glycerin, and citric acid as plasticizers. The physicochemical properties of the starch and the gelatinized solutions were characterized. The effect of different ratios of starch, glycerin, and citric acid over the mechanical and barrier properties of plantain starch films was also evaluated and compared with a previous report in achira starch films under similar conditions (Ávila Martín, 2018). The potential use of the plantain starch-based films in packaging is discussed based on the found results.

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# **1. Methods and materials**

## **1.1 Reagents**

Plantain starch from plantain flour of the *Musa-paradisiaca* Dominican-Harton variety grown in the department of Casanare. Distilled water, USP glycerin provided by White Chemical S.A.S (Bogota, Colombia), and Citric acid monohydrate from Merk (Germany) were used.

## **1.2 Methods**

### **1.2.1 Starch isolation**

Plantain starch was obtained by dry method from green plantain flour of the Dominican variety hartón, using a mechanical sieve RO-TAP brand with a series of six ASTM sieves. Batches of 500 grams of flour were taken and sieved for 5 minutes, then the samples of starch retained in the screen No. 325, were labeled and stored in airtight plastic bags (Mazzeo et al., 2008).

### **1.2.2 Granule size distribution**

Starch samples were suspended in industrial grade Ethanol (96%) and the particle size distribution of starch granules was determined using a Master Sizer 2000 (Malvern, UK) by Dynamic Light Scattering. Measurements were done at 25°C and sonication was used before and during the analysis (Núñez-Santiago et al., 2004).

### **1.2.3 Transmitted light microscopy and polarized light**

Particle size distribution was also analyzed in an Olympus BX53M microscope. The starch sample was spread on a slide, and either mineral oil or water was added to

disperse the sample. A coverslip was placed for the analysis using transmitted light with magnifications at 10x, 20x, 40x, and 100x. Polarized light was used to determine the birefringence pattern. Data were analyzed by ImageJ software.

### 1.2.4 Scanning Electron Microscopy

The morphology of the starch granules was characterized using a Scanning Electron Microscope (FEI Quanta 200) operated in high Vacuum ( $3 \times 10^{-7}$  torr). The samples were observed in the environmental mode of the equipment without using any coating (Ávila Martín, 2018).

### 1.2.5 Moisture

Starch samples of 1 g were placed in a watch glass and the analysis was performed on an Adam Equipment AMB 50 moisture balance, at 105°C. The moisture content was also determined by weight loss as a function of temperature in a thermogravimetric analyzer (TGA).

### 1.2.6 Colorimetry

The whiteness index of starch was calculated using the CIE LAB model (Equation 1) with the  $a^*$ ,  $b^*$ ,  $L^*$  indexes obtained with a HunterLab spectrophotometer and the EasyMatch QC software. Measurements were made in three different samples.

$$WI = 100 - \sqrt{(100 - \bar{L})^2 + a^2 + b^2} \quad \text{Equation 1}$$

$L^*$ : Color lightness ( $L^*=0$  black and  $L^*=100$  white)

$a^*$ : Position between magenta and green. Positive values indicate magenta, and negative values indicate green.

$b^*$ : Position between yellow and blue. Positive values indicate yellow and negative values indicate blue.

### 1.2.7 Thermogravimetry

The thermogravimetric analysis of starch was carried out by using a Mettler Toledo TGA 1 SF/1100/268 instrument. The starch was analyzed in an alumina sample holder and the weight of the samples used was approximately 10 mg. Samples were heated from 30°C to 600°C under a nitrogen atmosphere at a flow rate of 50 ml/min. Four different heating ramps (1°C/min, 2°C/min, 5°C/min, and 10°C/min) were used to determine the kinetic parameters. The graphs were obtained and analyzed using the STARe System software, and the kinetic parameters were determined using the standard ASTM E1641.

### 1.2.8 Differential Scanning Calorimetry

For obtaining the starch gelatinization curve, a 1mg sample of starch were placed in a 40 $\mu$ L aluminum crucible and 10  $\mu$ l of distilled water was added. The crucible was then sealed and gently shaken. A Mettler Toledo DSC instrument was used for the analysis. The following temperature sequences were used: an isothermal ramp at 25°C for 5 minutes, then, a dynamic heating ramp from 25°C to 120°C at a heating rate of 10°C/min, all under a controlled atmosphere of nitrogen with a flux of 50 ml/min. For the endothermic peaks, the Initial Temperature ( $T_0$ ), Peak Temperature ( $T_p$ ), Final Temperature ( $T_c$ ), and the gelatinization enthalpy ( $\Delta H_p$ ) were obtained from the resulting thermogram using the STARe System software. For starch retrogradation analysis, the crucible was stored with the previously gelatinized sample at 4°C for seven days. After this period, an isothermal ramp was used at 4°C for 5 minutes, and then, a dynamic heating ramp was programmed at 10°C/min, from 4°C to 90°C using nitrogen atmosphere with a flux of 50 ml/min. The percentage of retrogradation was determined using the STARe System software.

### 1.2.9 Nuclear Magnetic Resonance

Samples for NMR were preprocessed as follows: 11 mg of starch were added to a vial, then, the sample was dried in an oven at 50°C for 3 hours, and, 0.5 ml of deuterated dimethyl sulfoxide were added. The sample was homogenized in a water bath at 70°C with vortex stirring and stored for 24 hours. Afterward, 28 microliters of trifluoroacetic acid were added. The sample was again homogenized using a vortex and transferred to a magnetic resonance tube. The measurement was performed in a Bruker Advance 400

MHz spectrometer. The conditions used in the test were 70°C and, 16 scans with an acquisition time of 4.78 seconds using a zg30 pulse (Schmitz et al., 2009).

The spectrogram was obtained with the Bruker TopSpin software version 4.0.7. To analyze the spectrogram, an apodization was performed; then, the Fourier transform was applied, and a first and second order adjustment were performed. The dimethyl sulfoxide residual at 2.5 ppm was taken as the reference peak, the peaks corresponding to the alpha-1,4 and alpha-1,6 bonds were detected manually, and the branching degree was calculated from equation 2.

$$DB(\%) = \frac{I_{\alpha-1,6}}{I_{\alpha-1,6} + I_{\alpha-1,4}} 100 \quad \text{Equation 2}$$

Where,  $I_{\alpha-1,4}$  and  $I_{\alpha-1,6}$  correspond to the value of the integrals of the peaks associated with the  $\alpha-1,4$  and  $\alpha-1,6$ .

### **1.2.10 X-ray Crystallography**

X-Ray diffraction patterns were measured in a diffractometer X-Pert PRO MPD (PANalytical) in order to determine the percentage of crystallinity of the native starch. The sample was analyzed in reflection mode at 30 Kv and 40 mA. The comminuted sample was placed in a thin layer on a glass sample carrier and exposed to Cu-K $\alpha$  radiation at a diffraction angle ( $2\theta$ ) from 10 to 30° with a scanning speed of 0.5°/min. The Bragg-Brentano geometry was used to perform the XRD measurements (Pelissari et al., 2012). The analysis of the diffraction patterns was carried out using the X'Pert Highscore 2.1.2 software (PANalytical). The percentage of crystallinity was determined using the software Origin2018 by subtracting the amorphous area from the total area, which is the result of the integration of the original curve and the baseline curve.

### **1.2.11 Fiber Content**

Two grams of sample were taken, and acid digestion was performed in 1.25% sulfuric acid solution for 30 minutes. Then, the sample was filtered and washed with boiling water until acid residues were removed. Next, basic digestion was performed with sodium hydroxide at 1.25% for 30 minutes. The sample was filtered again and washed with 96% ethanol. After washing, the excess sample was placed in a crucible and taken to an oven

at 90°C until constant weight. This analysis was performed at the laboratorio de poscosecha at Universidad del Quindío (Gibert et al., 2009).

### 1.2.12 Functional Properties

Two grams of starch were placed in a falcon tube, and 30 ml of distilled water at 30°C were added and homogenized. The tube was then immersed with the sample in a water bath at 30°C and kept there for 30 minutes. Afterwards, the sample was centrifuged at 5000 rpm for 20 minutes and filtered. The gel remaining on the filter paper was weighed, 10 ml of the supernatant were taken and dried out for 4 hours at 90°C (Olatunde et al., 2017). The weights were registered and the water absorption index (WAI), swelling power (SP), and water solubility index (WSI) were determined with the equations 3, 4, and 5, respectively. These analyses were also performed at the laboratorio de poscosecha at Universidad del Quindío.

$$WAI = \frac{\text{Gel weight}}{\text{sample weight}} \quad \text{Equation 3}$$

$$SP = \frac{\text{gel weight}}{\text{sample weight} - \text{solubles weight}} \quad \text{Equation 4}$$

$$WSI = \frac{\text{solubles weight}}{\text{sample weight}} \times 100 \quad \text{Equation 5}$$

### 1.2.13 Rheology

The rheological properties of the gels were measured on a rotational rheometer (Bohlin Instruments C-VOR 200, Malvern Instruments). The measurements were made using the Peltier cell in isothermal mode (65 °C±0.01), with a 4° and a 20-mm diameter cone-plate geometry (Vogelsang et al., 2014). The filmogenic suspensions were made before and were maintained at a temperature of 60°C until the start of the measurement.

### **1.2.14 Films preparation**

The films were prepared by solvent casting following the methodology proposed by (Ávila Martín, 2018). Aqueous starch suspensions were prepared (10% w/w), and heated up to 50°C, temperature at which glycerin was added. The heating was continued until a temperature of 90°C in the suspension was reached and was kept at that temperature for 10 minutes to guarantee the complete gelatinization. Mechanical agitation of 200 rpm was made during the whole process. Finally, the resulting gel was left to cool down to 60°C and was spread on acrylic plates. The plates were left to dry for two days at room temperature. Subsequently, the films obtained were removed from the support and stored in a watertight plastic bag for a week for further analysis.

All the films were prepared with a starch-water ratio of 10% w/w. Three sets of compositions were prepared to evaluate the effect of the addition of citric acid and glycerin over the physicochemical properties of the films. In the first one, the glycerin content was established at 30% w/w based on starch, and the citric acid addition was evaluated at 6%, 12%, 24% and 36% w/w starch basis.

The second set was to assess the variation of glycerin addition without citric acid (CA), and the glycerin content was evaluated at 10%, 20%, 40%, 60%, and 80% w/w. In the third set, the variation of glycerin was evaluated, setting the CA composition at 6% w/w, and the glycerin composition was assessed at 10%, 20%, 40%, 60%, and 80% w/w based on starch. For the preparation of the films with citric acid it was added at the same time as the starch. All the compositions were made in triplicate in order to evaluate replicability. The films were labeled as follows: first four characters correspond to the percentage of citric acid followed by the initials CA; the next five characters correspond to the percentage of glycerin followed by the initials GLY, so that 06CA30GLY is the label of a film with 6% of citric acid and 30% of glycerin.

### **1.2.15 Mechanical Properties**

The mechanical properties of films were measured as described by the ASTM D882-18, *Standard Test Method for Tensile Properties of Thin Plastic Sheeting*. A Shimadzu AG-IS Universal Testing Machine was used with 100 N load cell and rubber surface grips. The

calibrated length was 100 mm and the tensile speed was 5 mm/min. The laboratory presented temperature conditions of  $24 \pm 3^\circ\text{C}$  and relative humidity of  $49 \pm 7\%$ .

The samples were randomly cut from the cast film in the form of 10 x 150 mm rectangular specimens, presenting thicknesses between 0.13-mm and 0.14-mm. Thickness variations along each specimen were lower than 10%. Seven specimens were measured per composition. Force and displacement calculations, as well as the Young's modulus values, were determined using TRAPEZIUM 2.

### 1.2.16 Water Vapor Permeation

The test was performed according to ASTM E1653, using the wet-cup method in three different samples per composition. The cups were filled with distilled water, leaving 6 mm measured from the top edge, on which the film of equal diameter was mounted.

The cups were placed in a weathering chamber (LabTech, model LHT-0150E) at 50% RH and  $23^\circ\text{C}$ . Every 24 hours, for 21 days, their weight change was recorded using a Mettler Toledo precision balance.

For the calculation of water vapor permeability (WVP), the loss of mass of the cups was plotted as a function of elapsed time. The slope of the linear portion of this graph that best fits the line (regression coefficient,  $R^2$ , close to 1) represented the stabilization of water vapor diffusion through the films per unit time and was used in equation 6.

$$WVP = \frac{\Delta m \cdot e}{\Delta t \cdot A \cdot \Delta p} \quad \text{Equation 6}$$

Where:

$\Delta m/\Delta t$  : moisture loss weight per time unit ( $g/s$ )

$A$ : film area exposed to moisture transfer in ( $m^2$ )

$e$ : film thickness

$(m)$  and  $\Delta p$  - water vapor pressure difference between the two sides of the film ( $Pa$ ).

### **1.2.17 Statistical analysis**

Analysis of variance (ANOVA) using Tukey's multiple comparisons test were performed to compare test results with three variables using the GraphPad Prism 8 Software, statistical differences were considered when  $p \leq 0.05$ .

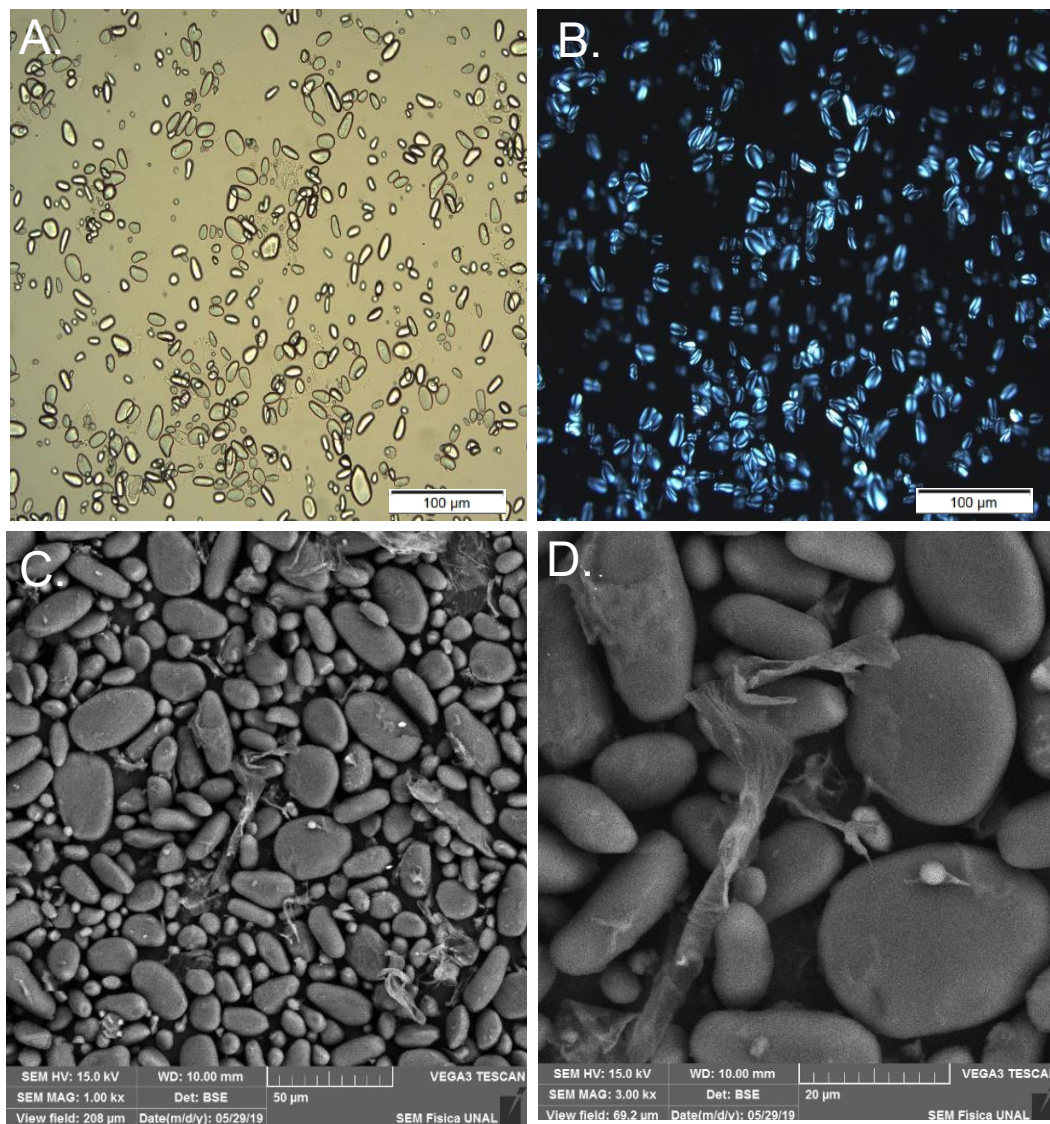
## **2.Results and discussion**

### **2.1 Starch Characterizations**

#### **2.1.1 Physicochemical properties**

The starch sample observed between cross polarizers shows the Maltese cross (Figure 1-B), which is characteristic of starch granules (Nakamura, 2015). Granules with heterogeneous sizes and shapes are seen in the sample, mainly of lenticular morphology, uniform surface without defects or pores (Figure 1-C). This distribution of shapes is consistent with previous reports (Chávez-Salazar et al., 2017). Also, in Figure 1-D, the presence of fiber in the sample can be seen, which can be associated with residuals from the extraction method.

The amount of fiber present in the sample was quantified and correspond to 0.46%, which is in line with the 0.47% reported by (Olatunde et al., 2017) and almost doubles the 0.28% found by (Pelissari et al., 2012). In plantain starch there will always be a small percentage of non-starch polysaccharides, which are mainly associated with fiber and cellulose (Chávez-Salazar et al., 2017), the complete extraction of these components is possible, but it implies the use of multiple washing stages that can lead to sample losses, decreasing the extraction yield (Bemiller & Whistler, 2009).

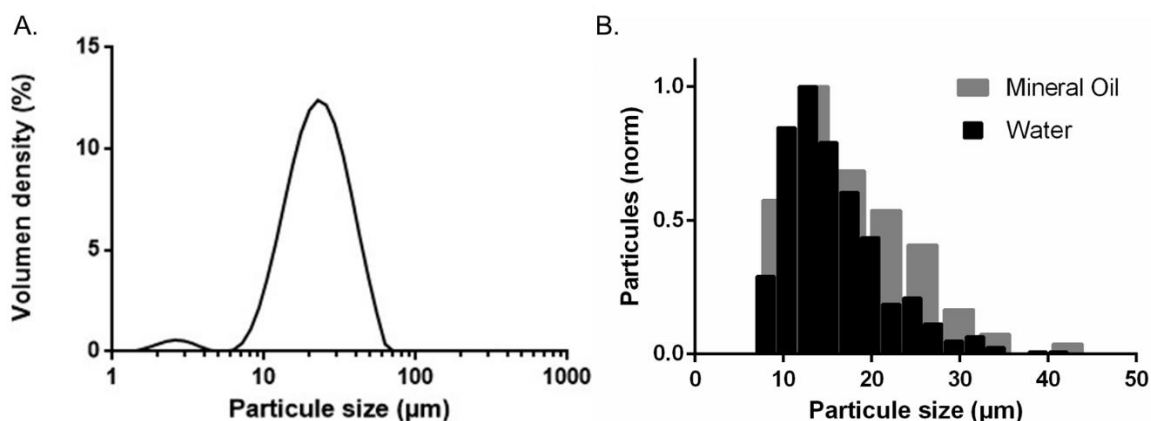


**Figure 1.** Plantain starch micrographs.

1A-(Micrograph, magnification 100X, scale bar=100 $\mu$ m), 1B-(Polarized light micrograph, magnification 100X, scale bar=100 $\mu$ m), 1C-(Scanning Electron Micrograph, magnification 1000X, scale bar=50  $\mu$ m), 1D-(Scanning electron micrograph, magnification 3000X, scale bar=20  $\mu$ m).

The particle size distribution measured by dynamic light scattering (DLS) is presented in Figure 2-A. A unimodal distribution is observed, with a very small peak at around 2.6  $\mu$ m. The main peak shows a median diameter of  $D [v; 0.5] = 22.3\mu\text{m}$  (the size at which 50% of the particles by volume are smaller and 50% are larger), 80% of the particles have a size range between 11.7  $\mu$ m and 39.8  $\mu$ m. The total size distribution range goes from 7.1  $\mu$ m

to 71  $\mu\text{m}$ . The size distribution is consistent with the values reported by (Núñez et al., 2004) and (Pelissari et al., 2012) who found a value of  $D [v; 0.5] = 24.31 \mu\text{m}$  and a size range from 7  $\mu\text{m}$  to 70  $\mu\text{m}$  and 3.3  $\mu\text{m}$  to 88  $\mu\text{m}$  respectively. The particle size distribution is mainly due to the method of extraction, in this work the sample was passed through the mesh No. 270, which corresponds to a pore size of 56  $\mu\text{m}$ . A starch sample with a larger particle size may have been retained.



**Figure 2.** Particle size distribution of plantain starch.

2-A Particle size distribution measure performed in Mastersizer analyzer, using ethanol as solvent. 2-B Particle size distribution measure performed in ImageJ from the image taken with an Optical microscope in water (black) and mineral oil (gray).

On the other hand, Figure 2-B presents the particle size distribution obtained by measuring the sizes from optical micrographs. It can be seen that measured diameters are lower than those obtained by DLS. Also, Figure 2-B compares the PSD measurement using water and mineral oil, as solvents. It can also be seen that no significant changes in the size distribution were found when changing the solvents. In this research, all results are reported with water as the dispersant media in optical microscopy.

### 2.1.2 Functional properties

The whiteness index of plantain starch was determined and compared with the value found for other native starches studied previously by the research group of Procesos Químicos y Bioquímicos of the Universidad Nacional de Colombia and are reported in the Table 1. The value of  $L^*$ , is similar to the value reported by (Pelissari et al., 2012),  $90.9 \pm$

0.4. Concerning the other native starches, it is observed that plantain starch has the highest yellowish color, this may be due to the lack of use of antioxidant agents during the extraction process. Further purification of the starch should be carried out to obtain a whiter product; however, the additional cost overruns on a large scale should be evaluated.

**Table 1.** Color parameters of different native starches determined by spectrophotometry

	<b>L*</b>	<b>a*</b>	<b>b*</b>	<b>WI</b>
<b>Maize</b>	96.9 ± 0.1	0.40 ± 0.01	4.99 ± 0.03	94.1 ± 0.1
<b>Achira</b>	86.7 ± 0.2	1.13 ± 0.02	9.01 ± 0.05	83.9 ± 0.2
<b>Balu</b>	89.0 ± 0.1	0.12 ± 0.02	8.28 ± 0.19	86.2 ± 0.1
<b>Plantain</b>	88.4 ± 0.9	1.10 ± 0.04	12.5 ± 0.19	82.9 ± 0.1

The swelling power is the capacity of the granules to absorb water under excess water conditions and takes higher values as the temperature increases. Due to the hygroscopic character of starch, granules can increase its diameter up to 3 times by swelling (Bemiller & Whistler, 2009). The values in Table 2 were determined at 50 °C, authors as (Olatunde et al., 2017) found values for SP ranging from 1.38 g/g to 2.14 g/g for native and modified plantain starches, while (Pelissari et al., 2012) found an SP value of 2.4 g/g and a WSI value of 1.5%.

**Table 2.** Functional properties of plantain starch

<b>PROPERTY</b>	<b>DATA</b>
Whiteness Index (WI)	82.9±0.1
Swelling Power (SP) g/g	2.11
Water Absorption (WAI) g/g	2.05
Water Solubility Index (WSI) %	2.5

### 2.1.3 Crystallinity

Diffraction patterns obtained by XRD are characteristic of the type of crystals present in starch. XRD patterns do not indicate that all those starches with a specific morphology present the same diffraction pattern. Still, they can be grouped according to their physical properties, for example, some cereal starches have a type A-crystal pattern, tuber and amylose rich cereal starches are usually characterized by a B-type pattern. In contrast, some legume starches generally have a C-type pattern, which one is a mixture of A-type and B-type crystalline orders (Bemiller & Whistler, 2009).

The type of crystal formed by the double helix organization was determined by XRD in Figure 3, compares the XRD patterns for plantain, maize, balu, and achira starches. For plantain starch, the peaks were presented at  $2\theta=15.14$ ,  $2\theta=17.2$ ,  $2\theta=18$  and  $2\theta=23.4$ , similar to the ones reported by (Ramirez-Cortes et al., 2016) which are characteristic of C-type pattern crystals. The degree of crystallinity found was 41%. This value of crystallinity is almost double that reported by (Pelissari et al., 2012) who reported a value of 22.8% and (Páramo-Calderón et al., 2016) who reported values between 22.35% and 26.6%, while (Bello-Pérez et al., 2005) reports a value of 36%. All authors report C-type crystallinity pattern, specifying type C<sub>B</sub>, which corresponds to a higher percentage of B-type crystals in the mixture. Regarding achira, balu, and corn starches, the crystallinities found were 22.0%, 28.1%, and 42.1%, and presented crystallinity patterns type B, B, and A, respectively (Arroyave et al., 2020).

The starch as it has been previously mentioned is a semi-crystalline polymer, the amylopectin is mainly related to the crystalline part of the granule, since having branches it is organized in form of double helix what gives the organized form to the granule, these branches correspond around 5% of the molecule (Bertoft, 2017). The degree of branching found for plantain starch was  $1.93\pm 0.04\%$ ; (Arroyave et al., 2020) found values of  $2.77\pm 0.09\%$ ,  $1.55\pm 0.04\%$  and  $4.33\pm 0.05\%$ , for corn starch, achira and balu respectively.

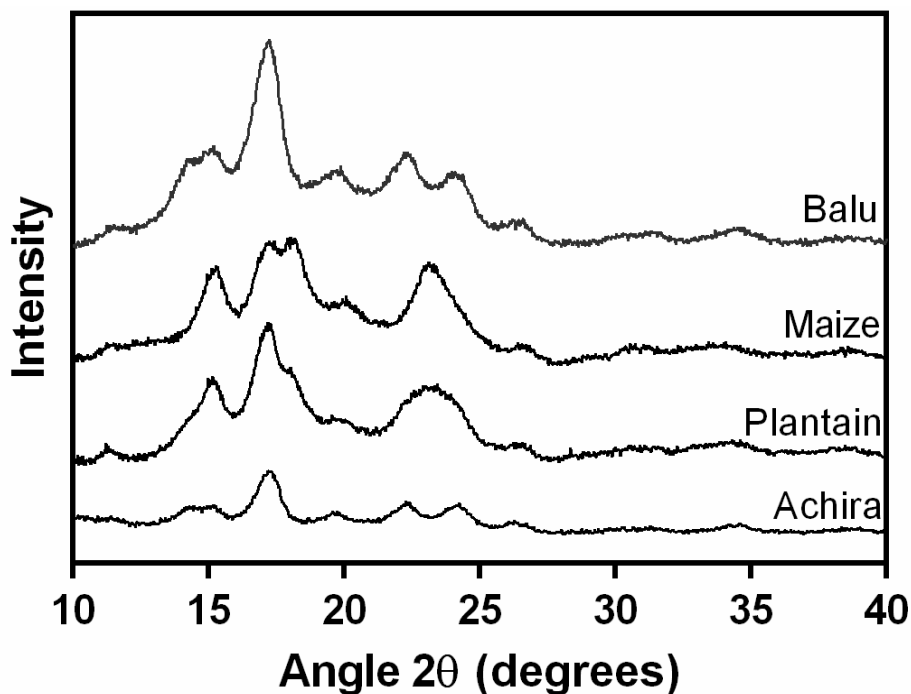
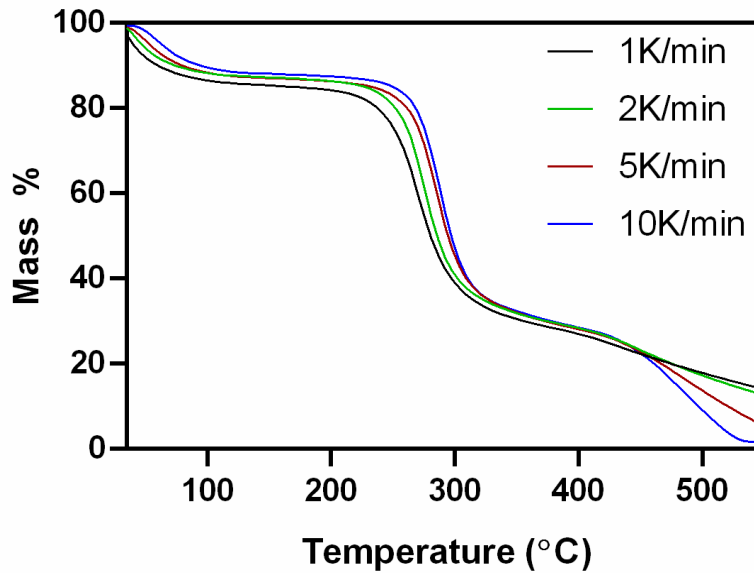


Figure 3. XRD patterns of four different native starches

#### 2.1.4 Thermogravimetry

The kinetics of starch degradation was studied by TGA. Four heating ramps (1°C/min, 2 °C/min, 5 °C/min, and 10 °C/min) were used between 35 °C and 600 °C as presented in Figure 4. It can be observed that in all the thermograms there are two main events of mass loss. First, between 40°C and 120°C a weight loss of 11%, which corresponds to the evaporation of moisture, is evidenced at the four heating ramps. A second event starting around 180°C and ending at about 410°C, is associated with covalent bond breaking generating volatile compounds with a loss of mass of 84%.



**Figure 4.** Thermal degradation of plantain starch, with various heating rates

According to ASTM E1641, the kinetics of thermal decomposition of polymers may be expressed in the form of equation 7.

$$\frac{d\alpha}{dt} = k(1 - \alpha)^n \quad \text{Equation 7}$$

Where  $\alpha$  is the fraction of material decomposed at time  $t$ ,  $n$  is the reaction order, and  $k$  is a kinetic constant. Combined with Arrhenius's empirical expression, it gives the following expression Equation 8:

$$\frac{d\alpha}{dt} = A e^{-E/RT} (1 - \alpha)^n \quad \text{Equation 8}$$

Where  $A$  is the pre-exponential or frequency factor,  $E$  corresponds to the activation energy,  $R$  the gas constant, and  $T$  is the absolute temperature. Introducing the heating rate  $\beta$ :

$$\frac{d\alpha}{dt} = \frac{A}{\beta} e^{-E/RT} (1 - \alpha)^n \quad \text{Equation 9}$$

Several solutions have been proposed for the solution of Equation 9. Table 3 shows the mathematical models used in this study, and in Table 4, it appears the kinetic parameters for the thermal degradation of plantain starch.

**Table 3.** Mathematical models for determination of  $E_a$

Mathematical Model	Equation	Graphical parameters
Coast-Redfern (Modified) (CRm)	$\ln \left[ \frac{\beta}{T^2(1 - 2RT/E_a)} \right] = \ln \left[ \frac{-AR}{E_a \ln(1 - \alpha)} \right] - \frac{E_a}{RT}$	$\ln \left[ \frac{\beta}{T} \right] vs \frac{1}{T}$
Flynn-Wall-Ozawa (ASTM International, 2013b)	$\log \beta = \log \left[ \frac{AE_a}{Rg(\alpha)} \right] - 2.315 - 0.4567 \frac{E_a}{RT}$	$\log \beta vs \frac{1}{T}$
Kissinger-Akahira-Sunose (KAS)	$\ln \left[ \frac{\beta}{T_m^2} \right] = \ln \left[ \frac{AR}{E_a} \right] - \frac{E_a}{RT_m}$	$\ln \left[ \frac{\beta}{T_m^2} \right] vs \frac{1}{T}$

Activation energy is defined as the necessary energy for a change of state to occur, breaking, or modify the bonds distribution which limits the rate of reaction. The value found for the activation energy reported in Table 4, is slightly different from the value previously determined by (Pineda-Gómez et al., 2014) who found a value of 220.2 kJ/mol with a standard deviation of 3.9. Concerning other starch species, (Ávila Martín, 2018) reported an  $E_a$  value of 175.6 kJ/mol and 205 kJ/mol for achira and potato starch respectively, while (Naranjo, et al., 2020) reported  $E_a$  values of 201.9 kJ/mol, and 227.2 kJ/mol for balu and cassava respectively.

**Table 4.** Kinetic parameters of plantain starch

Model	$E_a$ (kJ/mol)	A (Ln A(min <sup>-1</sup> ))	n
Coast Redfern (modified)	226.57	43.99	-
Flynn-Wall-Ozawa (FWO)	226.59	46.14	-
Kissinger-Akahira-Sunose	226.57	47.83	0.39

### 2.1.5 Moisture

Starch is a highly hygroscopic material, and the moisture content is related to the type and amount of crystals, as well as the environmental conditions. The results for moisture content in plantain starch measured by thermobalance and thermogravimetry are compared in Table 5. It was anticipated that moisture content could be dependent on the measuring method. However, values of Table 5 are not significantly different from those reported by other authors such as (Núñez-Santiago et al., 2004), (Pelissari et al., 2012), (Sukhija et al., 2016) who report moisture values of  $9.9\pm 0.24\%$ ,  $9.27\pm 0.07\%$  and  $10.5\pm 0.19\%$  respectively.

**Table 5.** Plantain starch moisture

Method	Moisture (%)
Thermobalance	$11.42\pm 0.87$
TGA	$10.15\pm 1.02$

## 2.2 Gel characterizations

### 2.2.1 Differential Scanning Calorimetry

Starch gelatinization occurs in the presence of a solvent, usually water, and the application of heat. During gelatinization, water diffuses through the amorphous region, producing an initial swelling of the granule. With the action of heat, water disrupts the crystalline structures with the consequent loss of the birefringent patterns observed by polarized microscopy. The temperature at which this phenomenon occurs is called gelatinization temperature, which is a characteristic property of the specific starch. As gelatinization is a kinetic effect, the gelatinization temperature of a starch is a function of the solvent and its concentration. Additionally, some non-volatile solvents (i.e. glycerin) may plasticize the starch, facilitating its processing (Carvalho & Trovatti, 2015).

When storing the plasticized starch below its transition temperature, a phenomenon called retrogradation is observed, which consists on the recovery of the crystalline structure. This process occurs due to the natural decrease in the energy-thermodynamic level. The percentage of retrogradation that the plasticized starch may have depends on the

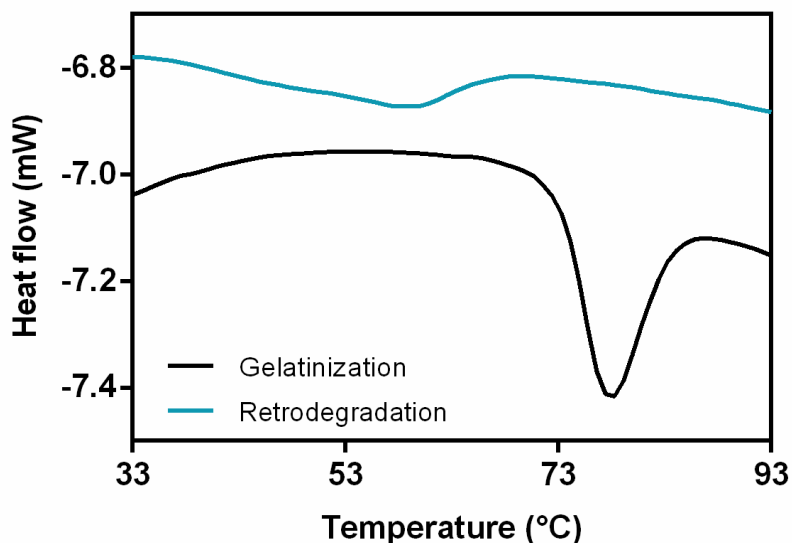
amylose/amylopectin ratio, temperature, water content, lipids, sugars, and the botanical source of the species as well as the plasticizer used (Fu et al., 2013).

The values of the determined gelatinization temperature and gelatinization enthalpy using water as a plasticizer can be found in the Table 6. The value of  $T_p$  for the same species found by (Páramo-Calderón et al., 2016) corresponds to  $79.82 \pm 0.15$  °C, and the Retrograde  $T_p$  is  $54.92 \pm 0.15$  °C. While some authors found  $T_p$  values for gelatinization of  $74.33 \pm 0.11$  °C (Chávez-Salazar et al., 2017),  $74.9 \pm 0.1$  °C (Pelissari et al., 2012), and  $80.7 \pm 0.21$  °C (Núñez-Santiago et al., 2004). In all cases, the gelatinization temperature values of plantain starch are above the gelatinization temperature of other starches, (Ávila Martín, 2018) reported a value of  $65.3$  °C,  $74.9$  °C,  $65.0$  °C and  $68.8$  °C for achira, corn, potato, and cassava starch, in accordance with this data (Zhang & Hamaker, 2012) reported values for  $T_p$   $71.3$  °C and  $66.8$  °C for corn and potato respectively.

**Table 6.** Thermal properties of plantain starch

<b>Gelatinization parameters</b>				
<b><math>T_0</math>(°C)</b>	<b><math>T_p</math> (°C)</b>	<b><math>T_c</math>(°C)</b>	<b><math>\Delta H_p</math> (J/g)</b>	<b><math>T_c - T_0</math></b>
$70.58 \pm 1.3$	$78.02 \pm 1.0$	$86.97 \pm 0.3$	$-12.50 \pm 0.5$	$16.39 \pm 0.98$
<b>Retrogradation parameters</b>				
<b><math>T_0</math>(°C)</b>	<b><math>T_p</math> (°C)</b>	<b><math>T_c</math>(°C)</b>	<b><math>\Delta H_p</math> (J/g)</b>	<b><math>T_c - T_0</math></b>
$37.99 \pm 1.9$	$58.6 \pm 0.97$	$67.91 \pm 1.6$	$-4.53 \pm 1.7$	$29.92 \pm 3.4$

$T_0$ : Onset temperature,  $T_p$ : Peak temperature,  $T_c$ : concluding temperature,  $\Delta H_p$ : gelatinization enthalpy.



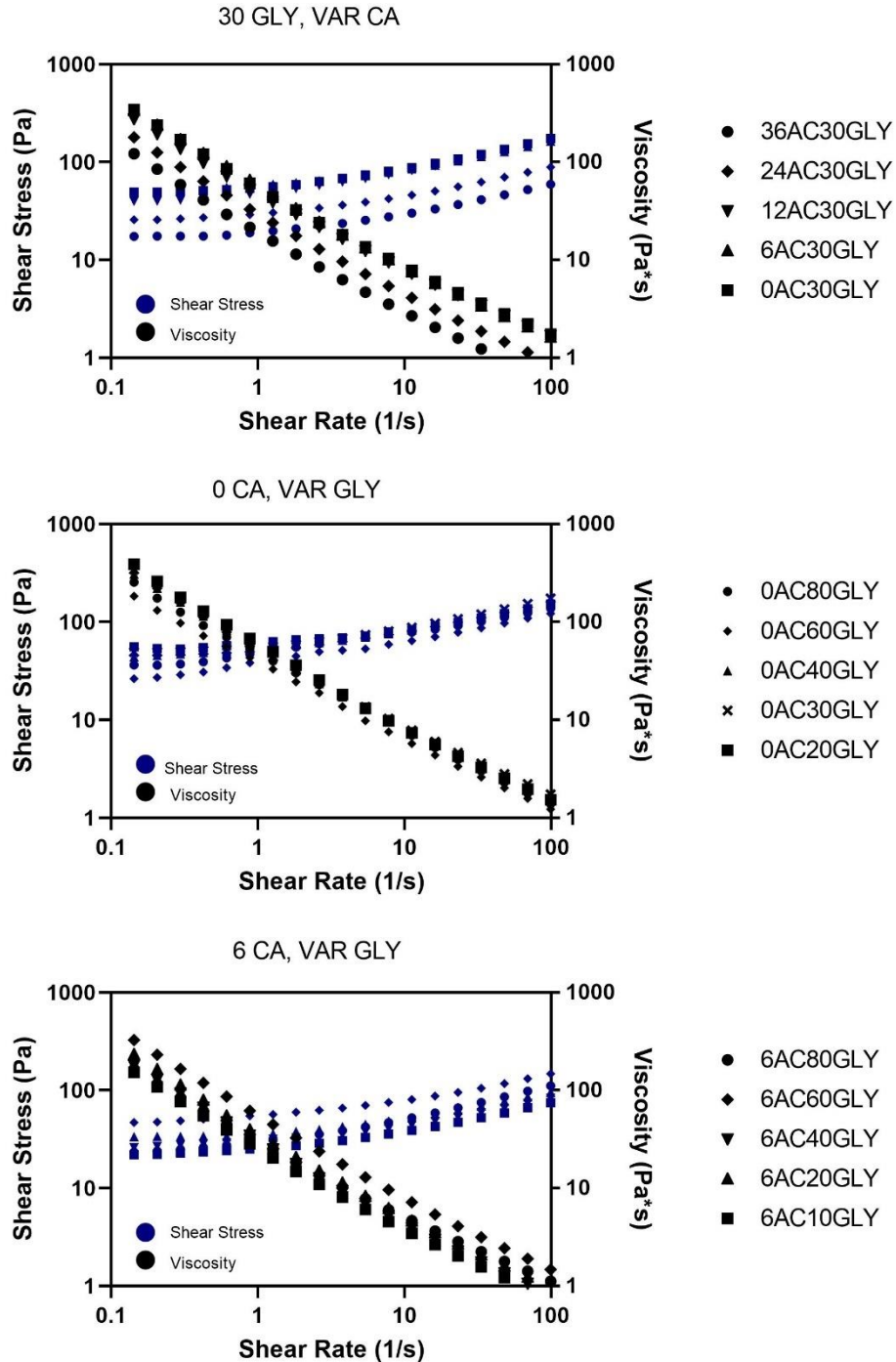
**Figure 5.** Differential Scanning Calorimetric of plantain starch

### 2.2.2 Rheology

Rheology is the science that studies the flow characteristics of a fluid under an external force (Wen, 2019). It is important to know the rheological properties of the starch gel since this helps to determine the behavior of the microstructure affected by the processing conditions. The flow behavior is determined to understand the ideal processing conditions, and to control the quality of the obtained product (Xie et al., 2012).

Figure 6 shows that the changes by glycerin addition do not considerably affect the viscosity of the gel. However, it can be seen that the lower the glycerin concentration, the higher the viscosity, this could be due to the fact that the lower the availability of the plasticizer the greater the number of granules available, which makes the fluid behave more like a filled polymer melt (Xie et al., 2012). Regarding to the addition of CA, the samples showed differences in viscosity since as its concentration increases, a decrease in viscosity is observed. But, the most considerable decrease is observed at CA concentrations above 12%. This behavior is consistent with the findings of (Ávila-Martín et al., 2020), who reports that CA forms higher hydrogen bonding interactions with starch than glycerol, and that weakens the interaction of starch molecules and improves its

plasticization. In fact, CA can depolymerize starch during processing, causing it to form shorter polymer chains (Xie et al., 2012).



**Figure 6.** Changes in the rheology of filmogenic solutions by varying the concentration of citric acid and glycerin

## 2.3 Films characterizations

For the evaluation of the film properties, three fixed variables were chosen to assess how the other involved variables influenced the mechanical and barrier properties of the film. In the first experiment, the concentration of glycerin was fixed at 30% w/w based on starch and the amount of citric acid was varied by 6%, 12%, 24%, and 36%. Whereas, for the second and third experiments, the citric acid concentration was fixed at 0% and 6%, respectively, and the glycerin concentration was varied by 20%, 40%, 60%, and 80%.

### 2.3.1 Mechanical properties

Mechanical properties are an essential feature in the classification of a starch film for use in packaging, since for this type of application the film is subjected to specific stresses. The determining property in the mechanical properties is the elastic modulus, it is the fundamental measure of the film stiffness, the higher the elastic modulus, the higher the stiffness of the material (Al-Hassan & Norziah, 2012).

In Table 7 are presented the results obtained for the mixtures studied. It is observed that as the CA concentration increase, the elastic modulus significantly decreases (ANOVA:  $p < 0.0001$ ;  $F = 36.8$  : Figure 7-A). However the main factor that impacts the decreasing of the elastic modulus is the presence of the CA, since only the 0CA30GLY is significantly different to the other compositions (Tukey's test:  $p < 0.0001$  between 0CA30GLY and each of the other compositions of the first experiment). These results can be explained due to the CA enters in the chains and improves their mobility, thus helping them to become more flexible (Versino et al., 2016), those results can also be observed in the second and the third experiments where the presence of CA diminishes the maximum value from 143.1 MPa (Figure 7-B) to 39.2 MPa (Figure 7-C) those results are consistent with that reported by (Jiugao et al., 2005).

In the second and third experiments it is observed that as the glycerin increase, elastic modulus significantly decrease, regardless of the absence (ANOVA:  $p < 0.0001$ ;  $F = 38.2$  Figure 7-B) or presence (ANOVA:  $p < 0.0001$ ;  $F = 20.6$  : Figure 7-C) of CA. This result can be due to the fact that the plasticizer helps to make the starch chains more flexible since it causes it to have a more disorganized structure, (Versino et al., 2016). It is also observed that a peak value is present in both experiments at a concentration of 30% of glycerin (Figure 7-B and Figure 7-B).

In Figure 7-D, it is observed that the tensile strength decreases as the CA content increases (ANOVA:  $p < 0.0001$ ;  $F = 73.41$ ). This behavior could be explained by the fact that CA may act as a crosslinking agent, improving the interaction between starch molecules and increasing the molecular weight. This interaction improves the tensile strength at low concentrations of CA. Nonetheless, as the degree of crosslinking increases, the tensile strength decreases because the chains lose mobility due to an increased interaction between the molecules (Reddy & Yang, 2010). It also presents a peak in the composition with a CA concentration of 6%, It could be that at that concentration, there is enough interaction to improve the tensile strength and increase the molecular weight, without losing the mobility of the starch chains, which happens at higher concentrations of CA. The observed trend, is similar to what is reported by (Ávila Martín, 2018) for achira starch, but the elongation percentages are higher in plantain starch.

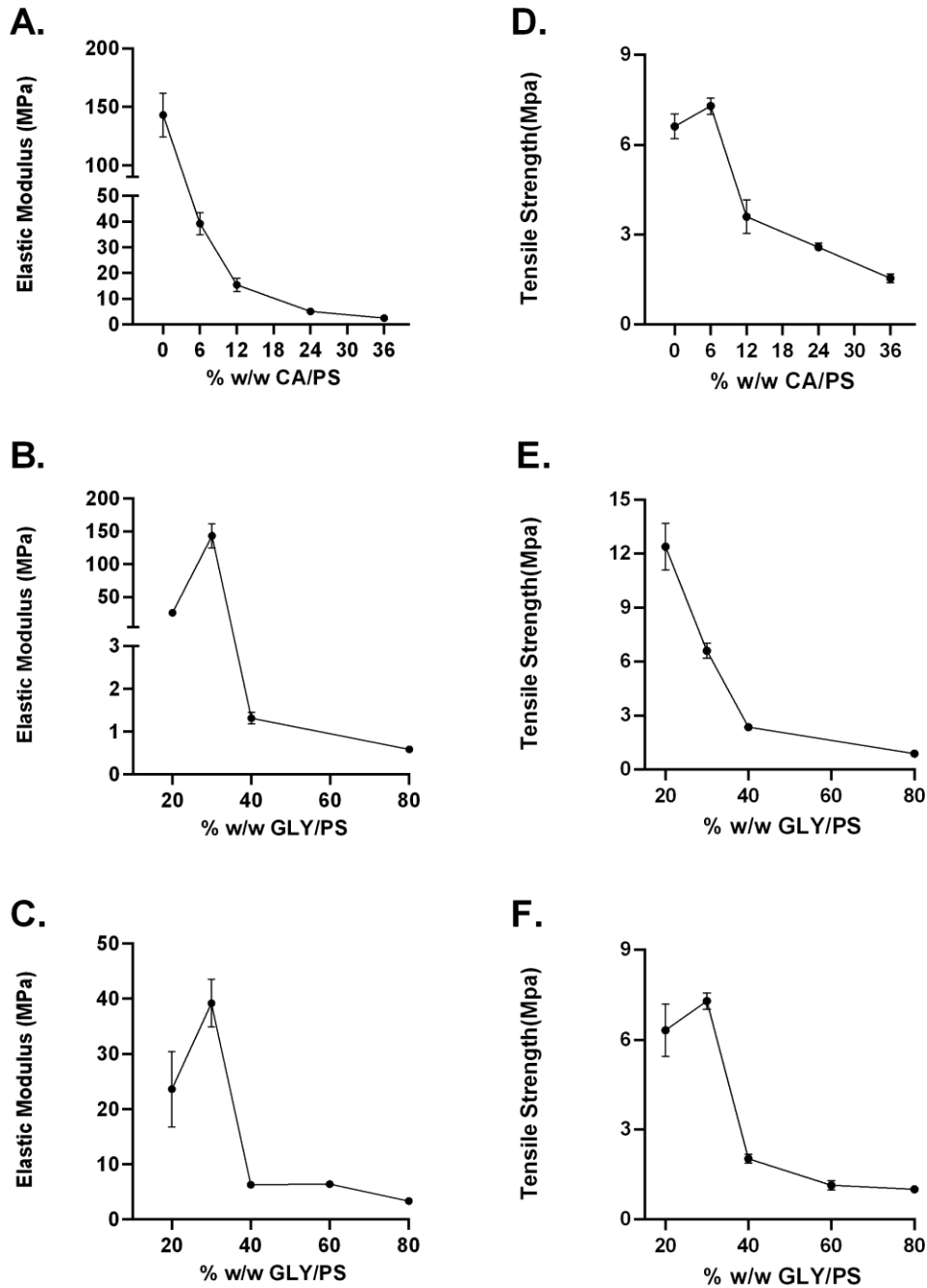
**Table 7.** Mechanical properties of plantain starch films with different compositions of citric acid and glycerin

Composition	Tensile Strength (MPa)	Elongation at break (%)	Elastic Modulus (MPa)
0CA30GLY	6.61 ± 0.9	15.2 ± 2.9	143 ± 41
6CA30GLY	7.29 ± 0.6	37.7 ± 6.6	39.2 ± 8.7
12CA30GLY	3.60 ± 0.8	40.1 ± 6.6	15.4 ± 3.7
24CA30GLY	2.58 ± 0.3	84.7 ± 13.1	5.11 ± 0.7
36CA30GLY	1.54 ± 0.3	88.8 ± 17.9	2.45 ± 0.20
6CA20GLY	6.32 ± 1.7	15.3 ± 5.2	23.6 ± 14
6CA40GLY	2.03 ± 0.3	41.9 ± 11.7	6.29 ± 1.1
6CA60GLY	1.15 ± 0.3	29.5 ± 9.1	6.41 ± 0.9
6CA80GLY	1.01 ± 0.1	43.9 ± 4.5	3.34 ± 0.1
0CA20GLY	12.4 ± 1.3	107.5 ± 3.2	25.8 ± 5.2
0CA40GLY	2.36 ± 0.3	295 ± 55	1.32 ± 0.3
0CA80GLY	0.89 ± 0.2	257 ± 56	0.59 ± 0.1

Regarding the effect of the concentration of glycerin over the tensile strength, it can be seen that the higher the glycerin concentration the lower the tensile strength, independently of the presence of CA (ANOVA:  $p < 0.0001$   $F = 137.1$  for CA=0% and  $p < 0.0001$   $F = 58.3$  for CA=6%). However, the presence of CA significantly decreased the tensile strength from 12.4 MPa to 6.32 MPa only in the composition with a concentration of 20% of glycerin (T test:  $p = 0.013$ ) for all the other concentrations of glycerin the CA

presence does not change the tensile strength significantly. It is because the glycerin makes the films elastic but less resistant to breakage.

The results showed that concentrations of glycerin higher than 30% have an elastic modulus and a tensile strength too low, making those very elastic materials but also very breakable. So, at a concentration of 30% of glycerin, a deformable and resistant material is obtained. Now from the first experiment, it was found that the composition with 6% of CA results in a material with a high tensile strength and a lower elastic modulus which means again a deformable and resistant material (Belibi et al., 2013). Those two characteristics are very useful, for example, in a flexible food packaging application, so the most suitable formula for this would be 6CA30GLY.

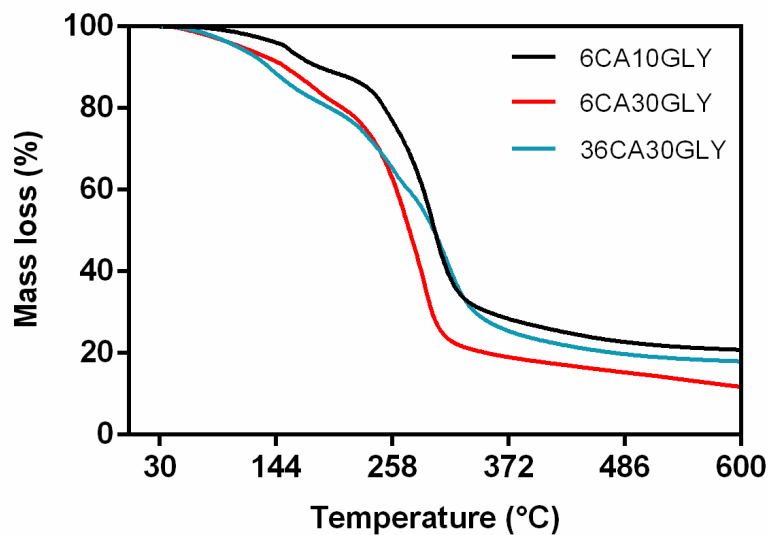


**Figure 7.** Mechanical properties for plantain starch films (PSF).

7-A. Elastic modulus for PSF at different concentrations of CA with 30% w/w Gly. 7-B. Elastic modulus for PSF at different concentrations of glycerin without CA. 7-C. Elastic modulus for PSF at different concentrations of glycerin using CA at 6% w/w. 7-D. Elastic tensile strength for PSF at different concentrations of CA with 30% w/w GLY. 7-E. Tensile strength for PSF at different concentrations of glycerin without CA. 7-F. Tensile strength for PSF at different concentrations of glycerin using CA at 6% w/w

### 2.3.2 Thermogravimetry

Three different combinations of starch films were subjected to thermogravimetric analysis to evaluate their degradability (06CA10GLY, 06CA30GLY and 36CA30GLY). Results are shown in Table 8 and Figure 8. It could be seen that as the concentration of CA increases the degradation temperature increases; this corresponds to the trend observed by (Ávila-Martín et al., 2020) with achira and maize starch, and may be explained because as the concentration of CA increases in the formulation, the starch-glycerin-CA interactions increases due to cross-linking (Reddy & Yang, 2010).



**Figure 8.** Effect of Citric Acid on thermal degradation of plantain starch films

Figure 8 shows two main mass losses, the first is due to the percentage of water present in the films, and the second is related to the degradation of the carbohydrate polymer. The film that showed a better degradability is the 06CA30GLY, with 15.3% residue, while for the 36CA30GLY the residue is 19.0%, and the 06CA10GLY a residue of 21.8%. Other authors such as (Ávila Martín, 2018) found for achira starch a percentage of degradation up to 10% lower than the results found for plantain starch. Which indicates that PS-based films are more easily degraded in the same conditions.

**Table 8.** Percentages of starch film degradation with Citric Acid

Composition	Degradation (%)	Degradation Temperature (°C)
6CA10GLY	78.2	297.7
6CA30GLY	84.7	290.3
36CA30GLY	81	312.5

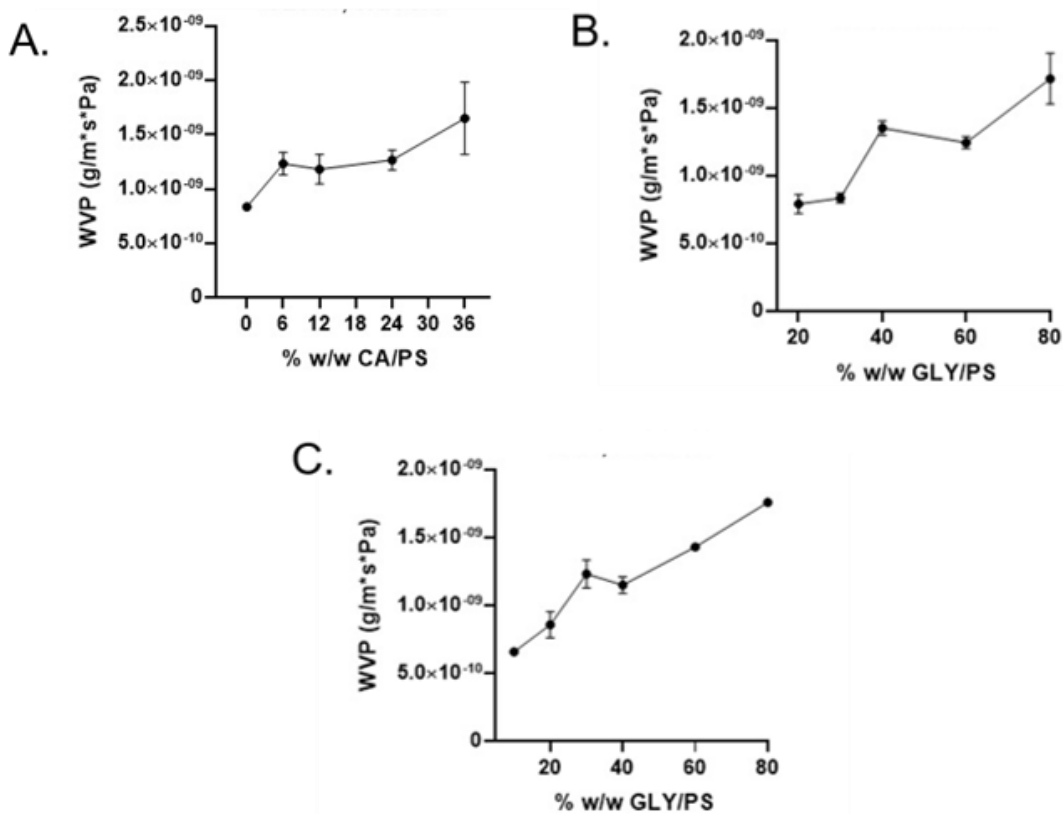
### 2.3.3 Barrier properties, WVP

A high WVP is one of the main disadvantages of starch-based films since due to its molecular structure presents high affinity to the water. It is also affected by the thickness of the film and the type of plasticizer used in the formulation. For films that do not have a high concentration of plasticizer, present better barrier properties as seen on Figure 9. However, these films are fragile and rigid. In this way, when increasing the plasticizer concentration, the mobility of the polymer chains is also increased, thus improving its elasticity and this contributes to decreasing the barrier properties of the films (Bertuzzi et al., 2007).

**Table 9.** Water Vapor Permeation for plantain starch films at different compositions

Composition	Water vapor permeability, WVP (10 <sup>9</sup> ) (g/m·s·Pa)
0CA30GLY	0.84 ± 0.05
6CA30GLY	1.23 ± 0.15
12CA30GLY	1.18 ± 0.19
24CA30GLY	1.27 ± 0.13
36CA30GLY	1.65 ± 0.47
6CA20GLY	0.91 ± 0.16
6CA40GLY	1.15 ± 0.10
6CA60GLY	1.43 ± 0.03
6CA80GLY	1.76 ± 0.05
0CA20GLY	0.79 ± 0.12
0CA40GLY	1.35 ± 0.09
0CA60GLY	1.24 ± 0.06
0CA80GLY	1.72 ± 0.27

The obtained results for the WVP can be found in the Table 9. The values obtained in the first experiment are similar to the values published by (Ávila-Martín et al., 2020) in achira starch based films. That indicates that CA has an effect over the barrier properties of the film regardless the botanical source of the starch.



**Figure 9.** WVP curves for plantain starch films at different concentrations of citric acid and glycerin.

9-A. WVP of plantain starch films at different concentrations of CA with 30% w/w Gly.

9-B. WVP of plantain starch films at different concentrations of glycerin without CA.

9-C. WVP of plantain starch films at different concentrations of glycerin with 6% w/w CA

In the first experiment CA concentrations tends to increase the WVP, but this trend is not statistically significant (ANOVA:  $p=0.16$ ,  $F=2.13$ ) (Figure 9-A), these results are consistent with what is reported by (Ávila-Martín et al., 2020) in achira starch based films. On the other hand, glycerin concentrations significantly increase the WVP regardless of the

presence (ANOVA:  $P < 0.0001$ ,  $F = 26.7$ ; Figure 9-C) or not (ANOVA:  $p = 0.0002$ ,  $F = 15.8$ ; Figure 9-B) of CA. This is because glycerin increases the amount of available OH groups, so the amount of water that can be retained by the film increases. Similar results were reported by (Bertuzzi et al., 2007) for other starches. Compared to the work of (Ávila Martín, 2018), it is observed that plantain starch presents similar barrier properties than achira starch at the same concentrations

## **3. Conclusions and recommendations**

### **3.1 Conclusions**

Plantain starch can be easily obtained by the dry method, nevertheless it contains higher fiber content and a yellowish color.

The viscosity of the plantain starch gel can be modified by changing the concentration of CA, but it is not affected by the concentration of glycerin.

The concentration of CA and/or of glycerin are inversely correlated with the elastic modulus and the tensile strength parameters.

The flexibility of the film can be improved by increasing the concentrations of CA and of glycerol. However, it also implies to make the film less resistant to breakage, so finding the best formula will depend on the desired application.

The degradation temperature of the film can be increased by the addition of higher concentrations of CA, but it does not change the degradation percentage significantly.

The addition of higher concentrations of either CA or glycerol increase the WVP, declining the barrier properties of the plantain starch-based films.

The selection of the right concentrations of plasticizers should be based on the special needs of the application, for example the 06CA30GLY film has characteristics that could be acceptable for an application in flexible packaging for food.

Plantain starch-based films have higher elongation at break percentages than achira starch-based films.

Plantain starch is an inexpensive, widely available raw material and can be used to make films that can replace single-use plastics.

## **3.2 Recommendations**

Enough starch must be isolated for the entire investigation to avoid batch variability.

In the starch extraction process, bleaching agents can be added to improve the physical appearance of the films.

In order to have a complete disruption of the starch granule, temperatures up to 10 °C above the gelatinization temperature must be reached, and enough time must be guaranteed for this process to take place.

Mechanical characterization tests should always be carried out at the same time after the film preparation, since over time the film presents retrogradation, and it makes some of its elastic properties to decrease.

### **3.2.1 Future research**

To study the effect of plasticizers over the barrier properties of the films with respect to other gases such as O<sub>2</sub> and CO<sub>2</sub>.

To evaluate the CA role in the filmogenic solutions, in order to determine if its main interaction is as plasticizer, crosslinker or hydrolyzer.

To study the length of the branched chains in order to determine its relationship with the crystallinity in the starch.

To study the relationship between the crystallinity and the mechanical properties of the films at different time points after manufacturing.

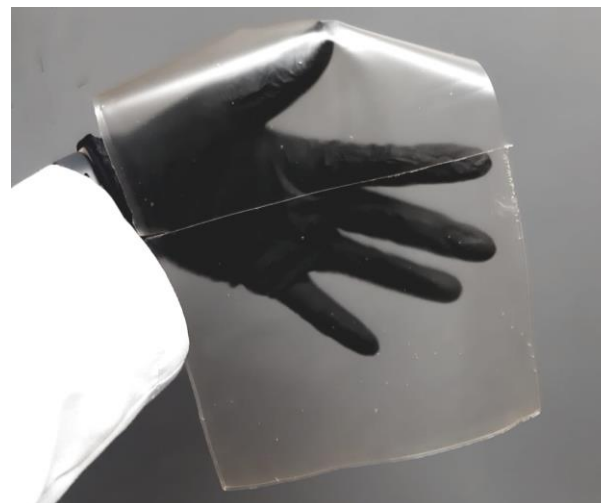
Testing concentrations between 25% and 35% w/w of glycerin and between 3% and 9% w/w of CA, in order to better characterize these specific range of concentrations at which some of the mechanical and barrier properties have a particular behavior.

To carry out tests in tape casting to obtain a product at a pilot scale.





## A. Annex: Set-up for plantain-starch films preparation





## 4. References

- Al-Hassan, A. A., & Norziah, M. H. (2012). Starch-gelatin edible films: Water vapor permeability and mechanical properties as affected by plasticizers. *Food Hydrocolloids*, 26(1), 108–117. <https://doi.org/10.1016/j.foodhyd.2011.04.015>
- Arroyave, S., Avila-Martin, L., Naranjo, E., & Perilla, J. E. (2020). Functional properties and physicochemical characterization of native starch from Andean crops for the production of sustainable biomaterials. *2020 AIChE Annual Meeting*.
- ASTM International. (2013a). *D1653-13 Standard Test Methods for Water Vapor Transmission of Organic Coating Films*. <https://doi.org/10.1520/D1653-13.2>.
- ASTM International. (2013b). *E1641-16: Standard Test Method for Decomposition Kinetics by Thermogravimetry Using the Ozawa / Flynn / Wall Method 1*. <https://doi.org/10.1520/E1641-13.2>
- ASTM International. (2018). *D882-18: Standard Test Method for Tensile Properties of Thin Plastic Sheeting*, ASTM International. <https://doi.org/10.1520/D0882-18>
- Ávila-Martín, L., Beltrán-Osuna, Á. A., & Perilla, J. E. (2020). Effect of the Addition of Citric Acid and Whey Protein Isolate in *Canna indica* L. Starch Films Obtained by Solvent Casting. *Journal of Polymers and the Environment*, 28(3), 871–883. <https://doi.org/10.1007/s10924-019-01648-z>
- Ávila Martín, L. (2018). *Efecto de la adición de ácido cítrico y proteína de lactosuero en la elaboración de películas basadas en almidón de Canna indica L* (Universidad Nacional de Colombia). Retrieved from <https://repositorio.unal.edu.co/bitstream/handle/unal/68666/1015401865.2018.pdf?squence=1&isAllowed=y>
- Belibi, P. C., Daou, T. J., Ndjaka, J. M. B., Michelin, L., Brendlé, J., Nsomd, B., & Durand, B. (2013). Tensile and water barrier properties of cassava starch composite films reinforced by synthetic zeolite and beidellite. *Journal of Food Engineering*, 115(3), 339–346. <https://doi.org/10.1016/j.jfoodeng.2012.10.027>
- Bello-Pérez, L. A., De Francisco, A., Agama-Acevedo, E., Gutierrez-Meraz, F., & García-Suarez, F. J. L. (2005). Morphological and molecular studies of banana starch. *Food Science and Technology International*, 11(5), 367–372. <https://doi.org/10.1177/1082013205058409>

- Bemiller, J. N., & Whistler, R. L. (2009). *Starch: chemistry and technology* (Third). New York: Academic Press.
- Bertoft, E. (2017). Understanding starch structure: Recent progress. *Agronomy*, 7(3). <https://doi.org/10.3390/agronomy7030056>
- Bertuzzi, M. A., Castro Vidaurre, E. F., Armada, M., & Gottifredi, J. C. (2007). Water vapor permeability of edible starch based films. *Journal of Food Engineering*, 80(3), 972–978. <https://doi.org/10.1016/j.jfoodeng.2006.07.016>
- Biotec. (2020). *BIOTEC, one of the world's leading companies in the development and production of compostable bioplastics*. Retrieved from <https://en.biotec.de/about-us/company-film>
- Carvalho, A., & Trovatti, E. (2015). Biomedical Applications for Thermoplastic Starch. In S. Kalia & L. Averous (Eds.), *Biodegradable and Biobased Polymers for Environmental and Biomedical Applications* (pp. 14–34). Retrieved from <https://books.google.com.my/books?id=cP2OCwAAQBAJ&pg=PA58&lpg=PA58&dq=plant+fibers+can+be+subdivided+as+bast,+leaf,+seed,+and+cereal+straw+or+grass+fibers,+depending+on+their+origin&source=bl&ots=OY9vDQY3KK&sig=HFBUFKV6hMuSc0qXqaiDjByH530&hl=en&sa=X&redir>
- Chávez-Salazar, A., Bello-Pérez, L. A., Agama-Acevedo, E., Castellanos-Galeano, F. J., Álvarez-Barreto, C. I., & Pacheco-Vargas, G. (2017). Isolation and partial characterization of starch from banana cultivars grown in Colombia. *International Journal of Biological Macromolecules*, 98, 240–246. <https://doi.org/10.1016/j.ijbiomac.2017.01.024>
- Cova, A., Sandoval, A. J., Laredo, E., & Müller, A. J. (2009). EFECTO PLASTIFICANTE Y ANTI-PLASTIFICANTE DEL AGUA EN SISTEMAS A. *Suplemento de La Revista Latinoamericana de Metalurgia y Materiales*, S2(1), 45–46.
- Fu, Z. Q., Wang, L. J., Li, D., Zhou, Y. G., & Adhikari, B. (2013). The effect of partial gelatinization of corn starch on its retrogradation. *Carbohydrate Polymers*, 97(2), 512–517. <https://doi.org/10.1016/j.carbpol.2013.04.089>
- Gibert, O., Dufour, D., Giraldo, A., Sánchez, T., Reynes, M., Pain, J. P., ... Díaz, A. (2009). Differentiation between cooking bananas and dessert bananas. 1. morphological and compositional characterization of cultivated colombian musaceae (*Musa* sp.) in relation to consumer preferences. *Journal of Agricultural and Food Chemistry*, 57(17), 7857–7869. <https://doi.org/10.1021/jf901788x>
- Glenn, G. M., Orts, W., Wood, D. F., Glenn, G. M., Orts, W., Imam, S., ... Wood, D. F. (2014). Starch Plastic Packaging and Agriculture Applications. *U.S. Department of Agriculture: Agricultural Research Service*. Retrieved from <https://digitalcommons.unl.edu/usdaarsfacpub/1459%0AThis>
- Jiugao, Y., Ning, W., & Xiaofei, M. (2005). The effects of citric acid on the properties of thermoplastic starch plasticized by glycerol. *Starch/Staerke*, 57(10), 494–504. <https://doi.org/10.1002/star.200500423>

- Mazzeo, M., Alzate G, A., & Marín M, M. (2008). Obtención de almidón a partir de residuos poscosecha del plátano dominico hartón (*Musa aab simmonds*). *Vector*, 3, 57–69.
- Ministerio de Agricultura y Desarrollo rural. (2020). *Cadena de Plátano*. Retrieved from <https://sioc.minagricultura.gov.co/Platano/Documentos/004 - Documentos Competitividad Cadena/D.C. 2014 Octubre - Indicadores platano.pdf>
- Molavi, H., Behfar, S., Ali Shariati, M., Kaviani, M., & Atarod, S. (2015). A review on biodegradable starch based film. *Journal of Microbiology, Biotechnology and Food Sciences*, 04(05), 456–461. <https://doi.org/10.15414/jmbfs.2015.4.5.456-461>
- Nakamura, Y. (2015). *Starch: Metabolism and Structure*. <https://doi.org/10.1007/978-4-431-55495-0>
- Naranjo, E., Avila-Martin, L., Arroyave, S., & Perilla, J. E. (2020). Thermal behavior of starch from different botanical sources by thermogravimetric kinetic analysis. *GEP-SLAP 2020-2021*.
- Navia, D., Ayala, A., & Villada, H. (2015). Effect of Cassava Flour Gelatinization on Mechanical Properties of Bioplastics. *Biotecnología En El Sector Agropecuario y Agroindustrial*, 13(1), 38–44.
- Novamont. (2020). Mater-Bi. Retrieved July 4, 2020, from <https://www.novamont.com/eng/mater-bi#:~:text=Materials made of MATER-BI,than three Italian production facilities.>
- Núñez-Santiago, M. C., Bello-Pérez, L. A., & Tecante, A. (2004). Swelling-solubility characteristics, granule size distribution and rheological behavior of banana (*Musa paradisiaca*) starch. *Carbohydrate Polymers*. <https://doi.org/10.1016/j.carbpol.2003.12.003>
- Olatunde, G. O., Arogundade, L. K., & Orija, O. I. (2017). Chemical, functional and pasting properties of banana and plantain starches modified by pre-gelatinization, oxidation and acetylation. *Cogent Food & Agriculture*, 3(1), 1283079. <https://doi.org/10.1080/23311932.2017.1283079>
- Oliveira, J., Scheibe, A. S., Sereno, A., & Laurindo, J. B. (2013). Scale-up of the production of cassava starch based films using tape-casting. *Journal of Food Engineering*, 119(4), 800–808. <https://doi.org/10.1016/j.jfoodeng.2013.07.009>
- Páramo-Calderón, D. E., Carrillo-Ahumada, J., Juárez-Arellano, E. A., Bello-Pérez, L. A., Aparicio-Saguilán, A., & Alvarez-Ramirez, J. (2016). Effect of cross-linking on the physicochemical, functional and digestibility properties of starch from Macho (*Musa paradisiaca* L.) and Roatan (*Musa sapientum* L.) banana varieties. *Starch - Stärke*, 68(7–8), 584–592. <https://doi.org/10.1002/star.201500200>
- Pelissari, F. M., Andrade-Mahecha, M. M., Sobral, P. J. D. A., & Menegalli, F. C. (2012). Isolation and characterization of the flour and starch of plantain bananas (*Musa paradisiaca*). *Starch/Stärke*, 382–391. <https://doi.org/10.1002/star.201100133>

- Pineda-Gómez, P., Angel-Gil, N. C., Valencia-Muñoz, C., Rosales-Rivera, A., & Rodríguez-García, M. E. (2014). Thermal degradation of starch sources: Green banana, potato, cassava, and corn - kinetic study by non-isothermal procedures. *Starch - Stärke*, 66(7–8), 691–699. <https://doi.org/10.1002/star.201300210>
- Ramirez-Cortes, R., Bello-Pérez, L. A., Gonzalez-Soto, R. A., Gutierrez-Meraz, F., & Alvarez-Ramirez, J. (2016). Isolation of plantain starch on a large laboratory scale. *Starch/Staerke*, 68(5–6), 488–495. <https://doi.org/10.1002/star.201500272>
- Reddy, N., & Yang, Y. (2010). Citric acid cross-linking of starch films. *Food Chemistry*, 118(3), 702–711. <https://doi.org/10.1016/j.foodchem.2009.05.050>
- Schmitz, S., Dona, A. C., Castignolles, P., Gilbert, R. G., & Gaborieau, M. (2009). Assessment of the extent of starch dissolution in dimethyl sulfoxide by <sup>1</sup>H NMR spectroscopy. *Macromolecular Bioscience*, 9(5), 506–514. <https://doi.org/10.1002/mabi.200800244>
- Sukhija, S., Singh, S., & Riar, C. S. (2016). Isolation of starches from different tubers and study of their physicochemical, thermal, rheological and morphological characteristics. *Starch - Stärke*, 68(1–2), 160–168. <https://doi.org/10.1002/star.201500186>
- Valero-Valdivieso, M. F., Ortegón, Y., & Uscategui, Y. (2013). Biopolímeros: Avances y perspectivas. *DYNA (Colombia)*, 80(181), 171–180.
- Versino, F., Lopez, O. V., Garcia, M. A., & Zaritzky, N. E. (2016). Starch-based films and food coatings : An overview. *Starch*, 68, 1026–1037. <https://doi.org/10.1002/star.201600095>
- Vogelsang, D. F., Perilla, J. E., & Buitrago, G. (2014). Preparation of biopolymer films by aqueous tape casting processing. *Journal of Plastic Film & Sheeting*, 30(4), 435–448. <https://doi.org/10.1177/8756087914523440>
- Wen, X. (2019). Pocket Guide to Rheology: A Concise Overview and Test Prep for Engineering Students. In *Pocket Guide to Rheology: A Concise Overview and Test Prep for Engineering Students*. <https://doi.org/10.1007/978-3-030-30585-7>
- Xie, F., Halley, P. J., & Avérous, L. (2012). Rheology to understand and optimize processibility, structures and properties of starch polymeric materials. *Progress in Polymer Science (Oxford)*, 37(4), 595–623. <https://doi.org/10.1016/j.progpolymsci.2011.07.002>
- Zhang, P., & Hamaker, B. R. (2012). Banana starch structure and digestibility. *Carbohydrate Polymers*, 87(2), 1552–1558. <https://doi.org/10.1016/j.carbpol.2011.09.053>
- Zhang, P., Whistler, R. L., Bemiller, J. N., & Hamaker, B. R. (2005). Banana starch: Production, physicochemical properties, and digestibility - A review. *Carbohydrate Polymers*. <https://doi.org/10.1016/j.carbpol.2004.10.014>